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19 ABSTRACT (Continue on reverse if necessary and identify by block number) <p>'A novel transfer RNA gene of <u>Sulfolobus</u> has been cloned. It has a large "D-loop" containing 27 residues and appears to code for glutamic acid tRNA. The sequence CCGGU occurs twice within the "D-loop". Two symmetrical cuts within or just outside these sequences can produce a typical tRNA and an 18-base "intron". The gene occurs as a single copy in the genome and is transcribed. It is being determined that whether this "intron" is actually spliced out or it remains as a part of the mature tRNA.</p> <p>In addition, an approximately 9 Kb <u>EcoRI</u> genomic fragment of <u>Sulfolobus</u>, containing genes for two tRNA-methyltransferases has been cloned. The tRNAs isolated from <u>E. coli</u> cells containing this recombinant plasmid contain N²-methylguanosine (m²G) and 1-methyladenosine (m¹A), the modified nucleosides not normally present in <u>E. coli</u> tRNAs. It appears that some <u>Sulfolobus</u> genes are expressed when cloned in <u>E. coli</u> and at least some of its enzymes are functional in the <u>E. coli</u> environment.</p>			
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ANNUAL REPORT



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FULL TITLE: Structure and expression of various RNAs in the Archaebacteria.

ABBREVIATED TITLE: RNAs in Archaebacteria

INSTITUTION: The Board of Trustees of Southern Illinois University,
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Summary of Project Goals:

1. Separation and sequencing of various RNAs of thermophilic archaebacteria.
2. Sequencing of various tRNA genes of archaebacteria (mainly thermophiles) and their surrounding regions to determine the organization of these genes and to identify the potential transcription control regions and the transcript processing sites.
3. Identification of the transcription initiation and termination sites in tRNA genes of various archaebacteria.
4. Characterization of RNA processing in thermophilic archaebacteria, initially using small transcripts, e.g., tRNA gene transcripts.

Recent Accomplishments

We have partially sequenced specific regions of some of the previously produced clones containing Sulfolobus tRNA genes. Some of these tRNA genes contain introns while others do not. One of these genes appears to be novel. Its transcript can be folded into a tRNA-like structure, in which the "D-loop" contains 27 residues. It appears to code for tRNA^{Glu}_{CUC} and lacks the 3'-terminal CCA sequence of the mature tRNA. The sequence CCGGU occurs twice within the "D-loop"; the first four bases of which can pair with each other to form a stem. Two symmetrical cuts within or just outside these sequences (i.e., at any of the six pairs of positions) can produce a typical tRNA containing all of the invariant and semi-invariant residues at their respective positions and an 18-base "intron". The gene occurs as a single copy in the genome, as revealed by Southern hybridizations, using an "intron" and both the "5'- and 3'-exon" specific probes. The Northern hybridizations by the two "exon" specific probes indicated that the gene is transcribed. For some of the further studies we have subcloned this tRNA gene in pBluescribe vectors

(Stratagene) in such a way that T7 RNA polymerase produces the transcripts which contain the complete sequence of the tRNA from position 2 through 71 (of the mature tRNA), and all of the "intron" and, in addition, some extra sequences at the 5' and 3' ends derived from the multiple cloning sites of the vectors.

In an effort to study the synthesis of the modified nucleosides of tRNAs, we are cloning the genes for archaebacterial tRNA-modifying enzymes. We have isolated a recombinant plasmid containing an approximately 9 Kb EcoRI genomic fragment of Sulfolobus in the pUC 19 vector. This fragment seems to contain genes for two tRNA-methyltransferases. The nucleoside composition of the tRNAs isolated from IPTG (isopropylthiogalactoside) induced E. coli cells containing this plasmid, as analysed by Liquid Chromatography/Mass Spectrometry (LC/MS), revealed the presence of N²-methylguanosine (m²G) and 1-methyladenosine (m¹A) in these tRNAs. These two modified nucleosides are known to be present in Sulfolobus, but are not normally present in E. coli tRNAs. It appears that at least some of the thermophilic archaebacterial genes are expressed when cloned in E. coli and that at least some of their enzymes are functional in the E. coli environment.

We have also cloned several tRNA genes of Thermococcus. The structures of these genes are being analyzed.

Plans for next year:

During the remaining period of this contract, all of the above mentioned works will be continued.

Initially, we shall determine whether the "intron" sequence (18 bases) in the above mentioned tRNA^{Glu} gene is actually spliced out or whether it exists as part of the mature tRNA. If it is spliced out, then we shall try to determine how its splicing is different from splicing other introns.

Initially the insert of clone carrying m²G and m¹A modification activities will be gradually deleted from the ends to determine the minimum size of the insert required to express these activities. These genes and their flanking regions will be sequenced. These genes will then be fused, in phase, to the lac Z gene of the pUC 18 or 19 vector with an aim of producing large quantities of the enzymes required for further studies.

In addition, we shall start sequencing mature tRNAs of Sulfolobus. Initially, Glu isoacceptor will be sequenced to determine whether the above mentioned tRNA has the extra 18 base in the mature tRNA.

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